

AMENDMENTS TO THE CLAIMS

1. – 15. (Cancelled)

16. (Original) A method for separating one or more cells of a second type from a sample, said method comprising the steps of:

(a) introducing into one or more microfluidic channels (i) a sample comprising cells of at least a first and second type and (ii) a solution that preferentially lyses cells of the first type, to cause greater lysis of cells of the first type compared to cells of the second type;

(b) contacting the product of step (a) with a microfluidic device comprising obstacles disposed in a microfluidic channel, wherein said obstacles preferentially bind said second type of cell;

(c) collecting cells bound to said obstacles, thereby producing a cell population enriched in said second type of cell;

(d) arraying said cell population enriched in said second type of cell;

(e) identifying one or more cells of said second type in said population enriched in said second type of cell; and

(f) collecting said one or more cells of said second type, thereby separating said one or more cells of said second type from said sample.

17. (Original) A method for separating one or more cells of a second type from a sample, said method comprising the steps of:

(a) introducing into one or more microfluidic channels (i) a sample comprising cells of at least a first, second, and third type and (ii) a solution that preferentially lyses cells of the first type, to cause greater lysis of cells of the first type compared to cells of the second type;

(b) contacting the product of step (a) with a microfluidic device comprising obstacles disposed in a microfluidic channel, wherein said obstacles preferentially bind said third type of cell compared to said second type of cell;

(c) collecting cells not bound to said obstacles, thereby producing a cell population enriched in said second type of cell;

(d) arraying said cell population enriched in said second type of cell;

(e) identifying one or more cells of said second type in said cell population enriched in said second type of cell; and

(f) collecting said one or more cells of said second type, thereby separating said one or more cells of said second type from said sample.

18. (Original) A method for producing a population of cells enriched in a second type of cell, said method comprising the steps of:

(a) introducing into one or more microfluidic channels (i) a sample comprising cells of at least a first and second type and (ii) a solution that preferentially lyses cells of the first type, to cause greater lysis of cells of the first type compared to cells of the second type;

(b) contacting the product of step (a) with a microfluidic device comprising obstacles disposed in a microfluidic channel, wherein said obstacles preferentially bind said second type of cell; and

(c) collecting cells bound to said obstacles, thereby producing said population of cells enriched in said second type of cell.

19. (Original) A method for producing a population of cells enriched in a second type of cell, said method comprising the steps of:

(a) introducing into one or more microfluidic channels (i) a sample comprising cells of at least a first, second, and third type and (ii) a solution that preferentially lyses

cells of the first type, to cause greater lysis of cells of the first type compared to cells of the second type;

(b) contacting the product of step (a) with a microfluidic device comprising obstacles disposed in a microfluidic channel, wherein said obstacles preferentially bind said third type of cell compared to said second type of cell; and

(c) collecting cells not bound to said obstacles, thereby producing said population of cells enriched in said second type of cell.

20. (Cancelled)

21. (Original) A method for separating one or more cells of a second type from a sample, said method comprising the steps of:

(a) contacting a sample comprising cells of at least a first and second type with a microfluidic device comprising obstacles disposed in a microfluidic channel, wherein said obstacles preferentially bind said first type of cell compared to said second type of cell;

(b) collecting cells not bound to said obstacles, thereby producing a depleted cell population;

(c) arraying said depleted cell population;

(d) identifying said one or more cells of said second type in said depleted population; and

(e) collecting said one or more cells of said second type, thereby separating said one or more cells of said second type from said sample.

22. (Original) A method for separating one or more cells of a second type from a sample, said method comprising the steps of:

(a) contacting a sample comprising cells of at least a first and second type with a microfluidic device comprising obstacles disposed in a microfluidic channel, wherein said obstacles preferentially bind said second type of cell compared to said first type of cell;

(b) collecting cells bound to said obstacles, thereby producing a depleted cell population;

(c) arraying said depleted cell population;

(d) identifying said one or more cells of said second type in said depleted population; and

(e) collecting said one or more cells of said second type, thereby separating said one or more cells of said second type from said sample.

23. (Original) The method of any one of claims 16-22, wherein said second type of cell is a fetal red blood cell.

24. (Original) The method of any one of claims 16-20, wherein said solution in step (a) comprises NaHCO_3 and acetazolamide.

25. (Original) The method of any one of claims 16-20, further comprising the step, after step (a), of diluting the product of step (a) with a diluent in said one or more microfluidic channels.

26. (Original) The method of any one of claims 16-19, 21, and 22, wherein each of said obstacles is coated with a binding moiety.

27. (Original) The method of claim 16, wherein said binding moiety comprises an anti-CD71, an anti-CD36, an anti-GPA, or an anti-CD45 antibody, or a combination thereof.

28. (Original) The method of any one of claims 16, 17, and 20-22, wherein at least 75%, 80%, 90%, 95%, 98%, or 99% of said one or more cells of said second type in said sample are arrayed in said arraying device.

29. The method of any one of claims 18-19, wherein at least 75%, 80%, 90%, 95%, 98%, or 99% of cells of said second type in said sample are collected.

30. – 43. (Cancelled)

44. (Currently Amended) A method of producing a ~~depleted~~ cell population depleted or enriched in a second type of cell, said method comprising the steps of:

(a) contacting a sample comprising cells of at least a first and second type with a microfluidic device comprising obstacles that preferentially bind said first type of cell compared to said second type of cell; and

(b) collecting cells bound to said obstacles or collecting cells not bound to said obstacles, thereby producing a depleted or enriched cell population.

45. (Original) The method of claim 44, wherein said obstacles are coated with a binding moiety that binds to the surface of said first type of cell.

46. (Original) The method of claim 44, wherein said first type of cell is a fetal red blood cell.

47. (Original) The method of claim 44, wherein said second type of cell is a fetal red blood cell.

48. (Original) The method of claim 44, wherein at least 60% of cells of said first type in said sample are bound to said obstacles.

49. (Original) The method of claim 44, wherein at least 70% of cells of said second type in said sample are not bound to said obstacles.

50. (Original) The method of claim 44, wherein said obstacles are ordered in a two-dimensional array.

51. (Currently Amended) A microfluidic device for producing a cell population enriched or depleted in a second type of cell comprising obstacles disposed in a microfluidic channel, each of which ~~is coated with a binding moiety~~ comprises a fetal-cell specific, epithelial-cell specific, tumor-cell specific, stem-cell specific, bacteria specific, protozoan specific, or fungal specific antibody, wherein, when a sample comprising first and second types of cells is introduced into said device, said first or second type of cell preferentially binds to said obstacles.

52. (Original) The microfluidic device of claim 51, wherein said obstacles are ordered in a two-dimensional array.

53. (Original) The microfluidic device of claim 51, wherein each of said obstacles is between 50 and 500 μm high.

54. (Original) The microfluidic device of claim 51, wherein each of said obstacles has a dimension orthogonal to height that ranges between 5 and 500 μm .

55. (Original) The microfluidic device of claim 51, wherein each of said obstacles is disposed at least 10 to 1000 μm from any other obstacle.

56. (Currently Amended) The microfluidic device of claim 51, wherein said ~~binding moiety comprises~~ antibody is an anti-CD71, an anti-CD36, an anti-GPA, or an anti-CD45 antibody, or a combination thereof.

57. (Currently Amended) A microfluidic device for producing a cell population enriched or depleted in a second type of cell comprising:

(a) a first region of obstacles disposed in a microfluidic channel defining a fluid flow path, wherein said obstacles in said first region preferentially bind a first type of cell compared to a second type of cell; and

(b) a second region of obstacles disposed in said microfluidic channel, wherein said obstacles in said second region preferentially bind a third type of cell compared to a fourth type of cell,

provided that said first and third types of cells are not the same.

58. (Original) The microfluidic device of claim 57, wherein said second and said fourth types of cells are the same.

59. (Original) The microfluidic device of claim 58, wherein said second and said third types of cells are the same.

60. (Original) The microfluidic device of claim 57, wherein said first region and said second region are arranged in series with regard to fluid flow in said microfluidic channel.

61. (Original) The microfluidic device of claim 57, wherein said first region and said second region are arranged in parallel with regard to fluid flow in said microfluidic channel.

62. (Original) The microfluidic device of claim 61, wherein said obstacles in said first region are interspersed among said obstacles in said second region.

63. (Currently Amended) A microfluidic device for producing a cell population enriched or depleted in a second type of cell comprising:

(a) a first region of obstacles disposed in a microfluidic channel defining a fluid flow path, wherein said obstacles in said first region preferentially bind a first type of cell compared to a second type of cell, wherein said obstacles are arranged in at least two substantially parallel rows and in at least two substantially parallel columns; and

(b) a second region of obstacles disposed in said microfluidic channel, wherein said obstacles in said second region preferentially bind a third type of cell compared to a fourth type of cell, wherein said obstacles are arranged in at least two substantially parallel rows and in at least two substantially parallel columns,

wherein the first and second regions are disposed adjacent one another in a microfluidic channel, and wherein the rows in the second region are displaced relative to the rows in the first region by a distance of less than the distance between the rows in the first region.

64. (Original) The microfluidic device of 63, wherein the obstacles in the first region are coated with an anti-CD71, an anti-CD36, an anti-GPA, or an anti-CD45 antibody, or a combination thereof.

65. (Original) The microfluidic device of 63, wherein the obstacles in the second region are coated with an anti-CD71, an anti-CD36, an anti-GPA, or an anti-CD45 antibody, or a combination thereof.

66. (Original) The microfluidic device of claim 63, wherein the ratio of the distance between the rows in the first region to the distance between the columns in the first region is about $\sqrt{3}$.

67. (Original) The microfluidic device of claim 63, wherein the ratio of the distance between the rows in the second region to the distance between the columns in the second region is about $\sqrt{3}$.

Group VI

68. (New) A method for separating one or more cells of a second type from a sample, said method comprising the steps of:

(a) contacting said sample with a device comprising obstacles comprising a binding moiety that preferentially binds cells of a first type compared to cells of said second type; and

(b) releasing cells bound to said obstacles, thereby separating said cells from said sample.

69. (New) The method of claim 68, wherein said releasing in step (c) comprises applying a shear force or lysing said bound cells.

70. (New) The method of claim 68, further comprising arraying said cells after step (b).

71. (New) The method of claim 68, further comprising analyzing the cellular contents of said cells after step (b).

72. (New) The method of claim 71, wherein said analyzing comprises FISH.

73. (New) The method of claim 71, wherein said analyzing comprises nucleic acid analysis.

74. (New) The method of claim 68, wherein said first type comprises fetal cells, epithelial cells, tumor cells, stem cells, bacteria, protozoa, or fungi.

75. (New) The method of claim 68, identifying one or more cells of said first type after step (b).

76. (New) The method of claim 68, wherein said binding moiety comprises an antibody.

77. (New) The method of claim 76, wherein said antibody is a fetal-cell specific, epithelial-cell specific, tumor-cell specific, stem-cell specific, bacteria specific, protozoan specific, or fungal specific antibody.

78. (New) The method of claim 68, wherein said second type comprises white blood cells or red blood cells.

79. (New) A method for identifying one or more cells of a second type in a sample, said method comprising the steps of:

(a) contacting said sample with a device comprising obstacles comprising a binding moiety, wherein said binding moiety preferentially binds cells of said second type compared to cells of a first type; and

(b) staining cells bound to said obstacles to identify cells of said second type, wherein said second type is selected from the group consisting of fetal cells, epithelial cells, tumor cells, stem cells, bacteria, protozoa, and fungi.

80. (New) The method of claim 79, further comprising analyzing the cellular contents of said cells during or after step (b).

81. (New) The method of claim 80, wherein said analyzing comprises nucleic acid analysis.

82. (New) The method of claim 79, wherein said staining comprises FISH.

83. (New) The method of claim 79, wherein said second type comprises fetal cells, epithelial cells, or tumor cells.

84. (New) The method of claim 79, identifying one or more cells of said second type during or after step (b).

85. (New) The method of claim 79, wherein said binding moiety comprises an antibody.

86. (New) The method of claim 85, wherein said antibody is a fetal-cell specific, epithelial-cell specific, tumor-cell specific, stem-cell specific, bacteria specific, protozoan specific, or fungal specific antibody.

87. (New) The method of claim 79, wherein said first type comprises white blood cells or red blood cells.

88. (New) A kit comprising (i) a device of claim 51, and (ii) a lysis reagent capable of lysing said first or second type of cell bound to said obstacles.

89. (New) A kit comprising (i) a device of claim 51, and (ii) a stain for cells of said first or second type of cell bound to said obstacles.

90. (New) A kit comprising (i) a device of claim 51, and (ii) a reagent for nucleic acid analysis of cells of said first or second type of cell bound to said obstacles.

91. (New) A method of manufacturing a device for producing a cell population enriched or depleted in a second type of cell, said method comprising:

- (a) providing a substrate;
- (b) creating a plurality of obstacles on said substrate;
- (c) attaching a fetal-cell specific, epithelial-cell specific, tumor-cell specific, stem-cell specific, bacteria specific, protozoan specific, or fungal specific antibody to said plurality of obstacles; and
- (d) enclosing said plurality of obstacles to generate a fluidic channel.

92. (New) The method of claim 91, wherein the substrate is selected from a silicon-based substrate, a metal-based substrate, a silicon-on insulator substrate, a glass substrate or a polymeric-based substrate.

93. (New) The method of claim 91, wherein the creating step comprises the use of lithography, micromachining, casting, molding, embossing, wet chemical etch, dry chemical etch, milling, diamond cutting, electroforming, LIGA, or any combination thereof.

94. (New) The method of claim 91, wherein the pattern of obstacles is a two dimensional array.

95. (New) The method of claim 94, wherein the two dimensional array pattern comprises a plurality of rows wherein every subsequent row is shifted relative to a previous row.

96. (New) The method of claim 91, wherein the antibody is selected from the group consisting of anti-CD71, anti-CD36, anti-GPA, and anti-CD45.

97. (New) A fetal cell enrichment device comprising one or more binding moiety that selectively binds fetal cells or non-fetal cells and a matrix of one or more hydrogel polymers.

98. (New) A method for separating fetal cells from a sample comprising fetal and non-fetal cells comprising: flowing said sample through a matrix comprising a hydrogel polymer -containing a binding moiety that selectively binds fetal red blood cells or a non-fetal cell, thereby separating said fetal cells from a subset of said non-fetal cells.

99. (New) A tumor cell enrichment device comprising one or more binding moiety that selectively binds tumor cells or non-tumor cells and a matrix of one or more hydrogel polymers.

100. (New) A method for separating tumor cells from a sample comprising tumor and non-tumor cells comprising: flowing said sample through a matrix comprising a hydrogel polymer -containing a binding moiety that selectively binds tumor cells or a non-tumor cell, thereby separating said tumor cells from a subset of said non-tumor cells.

101. (New) A system comprising:
one or more size-based separation regions, wherein each of said size based separation regions comprise a plurality of obstacles that direct a first analyte of a fluid sample in a first direction and a second analyte of said fluid sample in a second direction, wherein said first analyte and second analyte have a different size; and
one or more capture regions that selectively captures either said first analyte, second analyte or a third analyte.

102. (New) A method for size-based separation of a first analyte from a second analyte of a different size in a fluid sample comprising:
applying said sample to one or more size-based separation regions and one or more capture regions, wherein each said size-based separation region comprises a plurality of obstacles which direct said first analyte in a first direction and said second analyte in a second direction and each said capture region selectively captures said first, second, or a third analyte.

103. (New) A device for producing a cell population enriched in a first cell type from a blood sample comprising a first inlet in communication with a channel comprising two rows of obstacles, wherein said two rows of obstacles direct said first cell type towards a first outlet in a first direction and a second cell type towards a second outlet in a second direction.

104. (New) A device for producing a cell population enriched in fetal red blood cells from a maternal blood sample comprising a first inlet in communication with a channel comprising a first plurality of gaps, wherein said first plurality of gaps directs said one or more fetal red blood cells towards a first outlet in a first direction and one or more maternal red blood cells towards a second outlet in a second direction.

105. (New) A device for producing a cell population enriched in epithelial cells from a blood sample comprising a first inlet in communication with a channel comprising a first plurality of gaps, wherein said first plurality of gaps directs said one or more epithelial cells towards a first outlet in a first direction and one or more non-epithelial cells towards a second outlet in a second direction.

106. (New) A method for producing a cell population enriched in a first cell type from a blood sample, said method comprising: applying said blood sample to a device having a first inlet in communication with a channel comprising a plurality of obstacles, wherein, when said blood sample flows through said channel, said plurality of obstacles direct said first cell type towards a first outlet in a first direction and a second cell type in said blood sample towards a second outlet in a second direction, thereby producing said cell population enriched in said first cell type.

107. (New) A method for producing a cell population enriched in fetal red blood cells from a maternal blood sample, said method comprising: applying said maternal blood sample to a device having a first inlet in communication with a channel comprising a plurality of obstacles, wherein, when said maternal blood sample flows through said channel, said plurality of obstacles direct said fetal red blood cells towards a first outlet in a first direction and one or more non-fetal red blood cells in said maternal blood sample

towards a second outlet in a second direction thereby producing said cell population enriched in fetal red blood cells.

108. (New) A method for producing a cell population enriched in epithelial cells from a blood sample, said method comprising: applying said blood sample to a device having a first inlet in communication with a channel comprising a plurality of obstacles, wherein, when said blood sample flows through said channel, said plurality of obstacles direct said epithelial cells towards a first outlet in a first direction and one or more non-epithelial cells in said blood sample towards a second outlet in a second direction, thereby producing said cell population enriched in epithelial cells.

109. (New) A method for producing a cell population enriched in tumor cells from a blood sample, said method comprising: applying said blood sample to a device having a first inlet in communication with a channel comprising a plurality of obstacles, wherein, when said blood sample flows through said channel, said plurality of obstacles direct said tumor cells towards a first outlet in a first direction and one or more non-tumor cells in said blood sample towards a second outlet in a second direction, thereby producing said cell population enriched in tumor cells.

110. (New) A device comprising a first array of obstacles disposed to selectively enrich, based on size, shape, or deformability, a first cell type having a concentration of up to 50×10^{-3} cells/ μl in a sample by a factor of at least 100,000.

111. (New) A device comprising a first array of obstacles that depletes one or more non-fetal cells from a maternal blood sample and recovers at least 90% of fetal cells present in said maternal blood sample.

112. (New) A device comprising a first array of obstacles that depletes one or more non-tumor cells from a blood sample and retains at least 90% of tumor cells in said blood sample.

113. (New) A method for producing a cell population enriched in a first cell type which is present in a sample at a concentration of up to 50×10^{-3} cells/ μ l comprising:
applying said sample to a device comprising a first array of obstacles to produce said cell population which is enriched in said first cell type by a factor of at least 100,000.

114. (New) A method for manufacturing a device for cell sorting comprising:
providing a substrate; and
creating a pattern of obstacles on said substrate, wherein said obstacles allow passage of cells based on their size, shape or deformability,
wherein the substrate comprises at least two outlets for collecting the sorted cells.